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THE EFFECT OF MIXTURE (SIDR INFUSION WITH HYDROGEN PEROXIDE) ON BACTERIA *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

The remarkable ability of *Pseudomonas aeruginosa* to adapt and thrive in wide variety of environments is due in part to its extensive genetic versatility, which contributes significantly to its potential pathogenicity. Depending on the environmental conditions and the immune status of the host, *P.aeruginosa* can be a quiescent colonizer, a case of chronic infections, or highly virulent invader during acute infections. The study is to assess the antibacterial effect of a new special solution made by a mixture of sidr infusion (Ziziphuss spina- Christi (L) var. inermis Boiss) with Hydrogen peroxide. In this study, *P.aeruginosa* isolates exhibited high resistance rate toward Cefataxime (CTX:30mg), Ceftazidme (Ca:30mg). Gentamicin (CN: 10 mg) and Carbenicillin (Py: 100mg). while Norfloxacin (NOR:10mg), Tobramycin (TOB:10mg) and Amikacin (Ami:30 mg) inbition zone of sensitive isolate in disc diffusion test were determined at (22.16 and 20) mm respectively. We was tested their decontamination using a special antibacterial solution; mixture (sidr leaves aqueous infusion 62.5 g\L with 1.5% Hydrogen peroxide). The mixture inhibition zone of sensitive isolate in well diffusion test was determined at 23mm. we concluded that special (mixture sidr infusion with hydrogen peroxide) antibacterial solution can eradicate *P.aeruginosa*.

KEYWORDS: Sidr Infusion, Hydrogen Peroxide, Special Antibacterial, *P.aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa was ranked as the most frequent pathogen in surgery[1]. This bacterium determines many kind of infections like urinary tract infection, respiratory system infections, dermatitis, soft tissue infections, bacterimia, bone and joints infections, gastrointestinal infections and a variety of systemic infections, particulary in immune suppressed patients with sever burns, cancer and AIDS. The pathogensis of *P.aeruginosa* is multifactorial, as suggested by a large number of cell associated and extracellular virulence determinants of this organism[2]. *P.aeruginosa* has capacity to adapt easily to change in the environment. It needs a minimal nutritional requirement to grow and rapidly develop resistance to antibiotics and produce arsenal of virulence[3]. The remarkable ability of *P.aeruginosa* to adapt and thrive in wide variety of environments is due in part to its extensive genetic versatility, which contributes significantly to its potential pathogenicity[4].

The first step in *P.aeruginosa* infections is the colonization of the altered epithelium, Adherence of *P.aeruginosa* to the epithelium is mediated by pili and flagella. Evidence also implicates LPS as an adhesive facter. *P.aeruginosa* produced viscous exopolysaccharaied (alginate) in upper airway of patient with cystic fibrosis. After colonization

P.aeruginosa produce several extracellular virulence facters pyocyanin, alkaline protease, elastase, protease IV, heat- labile and heat-stable hemolysine, neuraminidase and exotoxins A,S,U,Y,T responsible for extensive tissue damage, blood stream invasion and dissemination, Many of these extracellular virulence factors are controlled by cell to cell signaling system[5]. The biofilm formation allows *P.aeruginosa* to escape host defenses and resist the antimicrobial action of antibiotics[6]. Interaction between virulence factors and the host immune response determinate the severity and the type of infections[7]. Adding to the problems of high incidence and infection severity, the resistance of *P.aeruginosa* to conventional antimicrobial treatment has increased over the past decade[8]. It is important to stress that *P. aeruginosa* infections are difficult to treat because of the bacteria's intrinsic resistance to many antibiotics, owing to its low outer membrane permeability, and its ability to acquire new resistance mechanisms during antibiotic treatment[9].

The outer membrane of *P.aeruginosa* is responsible for the high resistance to many antimicrobial agents in comparison with other organisms in which there are some differences in (LPS) composition and in that cation content of the outer membrane[10]. The high Mg^{2+} content aids in producing strong Lps-Lps links. Furth more, the small size of pores may not permit general diffusion through them in to the cells[11]. Resistance to *β*-lactams in *P.aeruginosa* involves modification of the target site-pencillin binding proteins (PBP), altered PBP4 were reported after imipenem treatment, as well as after administration of high doses of piperacillin in patients suffering from cystic fibrosis[9]. Active efflux is an important non-enzymic mechanism of *β*-lactam resistance in *P. aeruginosa*. Efflux also contributes to the development of multiple resistances to all strategic antipseudomonal antibiotics[12] A high number of acquired resistance genes coding for ß-lactamases and aminoglycoside-modifying enzymes (AME) have been noted in *P. aeruginosa*. Extended-spectrum ß-lactamases have been increasingly reported and metallo-ß-lactamases have also started to emerge in *P.aeruginosa*[13]. Methylation of 16S rRNA has emerged a new mechanism of resistance against aminoglycosides among *P. aeruginosa*[14].

P.aeruginosa has one chromosome and it's genetic material is rich in guanine and cytocin, in addition to the presence of extra chromosomal genetic element called plasmid[15]. *Ziziphus spina-christi* commonly known as Christ's Thorn Jujube, is a deciduous tree and native to the warm-temperate and subtropical regions, including North Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and Middle East[16]. It belongs to the Rhamnaceae family in the order of Rosales that contains about 60 genera and more than 850 species. The genus *Ziziphus* consists of about 100 species of deciduous or evergreen trees and shrubs throughout the world[17]. *Z. spina-christi* has been among the key plants of the Iranian traditional medicine since ancient times and is indigenous and naturalized throughout Iran[18]. *Z. spina-christi* is a shrub, sometimes a tall tree, reaching a height of 20 m and a diameter of 60 cm; its bark is light-grey, very cracked, scaly; trunk twisted; very branched, crown thick; shoots whitish, flexible, drooping; thorns in pairs, one straight, the other curved. Its leaves are glabrous on upper surface, finely pubescent below, ovate-lanceolate or ellipsoid, apex acute or obtuse, margins almost entire, lateral veins conspicuous. Flowers in cymes, subsessile, peduncle 1 to 3 mm. Fruit about 1 cm in diameter[19]. *Z. spina-christi* has very nutritious fruits that are usually eaten fresh. The flowers are important source for honey in Yemen and Eritrea[20]. The fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery[17].

MATERIAL AND METHODS

Preparation of the Sidr tree-*Ziziphusspina-christi (L) var. inermis Boiss*, the fresh sidr leaves after being weighed as 62.5 g/L. They were cleaned from the dust through washing in tap water. One liter of distilled water was added to each

sample and heated up to boiling. The solution was left in the refrigerator for 12 hours. The remnants of the leaves were discarded, while the liquids infusion was mixed with 1.5% hydrogen peroxide to get the new invited antibacterial.

Disc diffusion test: the Kirby-bauer method is a standardized system that takes all variables into consideration. It is sanctioned by the United States FDA and the Subcommittee on Antimicrobial Susceptibility Testing of the NCCLS.[21]

- It was performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated *Pseudomonas aeruginosa* colonies grown on blood agar plates to 5 ml of nutrient broth, this culture was then incubated for 2 hrs. to produce a bacterial suspension of moderate turbidity that compared with turbidity of ready-made (0.5) McFarland tube standard provided by Biomerieux/ France. A sterile swap was used to obtain an inoculum from the standardized culture, this inoculum was then swapped on Mueller-Hinton plate.
- The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps, then incubated at 33C to 35C for a full 24 hrs. before reading the results to identify cells expressing heteroresistance[22]. Worthily for mentioning, the incubation period were modified to 16-18 hrs. when Cefataxime (CTX 30mg), Ceftazidme (Ca 30mg), Gentamicin (CN 10mg), Carbenicillin (Py 100mg), Norfloxacin (NOR 10mg), Tobramycin (TOB 10mg) and Amikacin (Ami 30mg) according to[23] recommandations.
- Antibiotics inhibition zones were measured using a transparence ruler. Zone size was compared to standard zones to determine the susceptibility of organism to each antibiotics[22].

Well diffusion method: In this method, Muller- Hinton agar plate was prepared by equally cutting spaced well (6mm), then the plates were inoculated with cotton swab dipping into scrow tube containing bacterial suspension *P.aeruginosa* and streaked over the surface of plates. After that, Muller-Hinton agar wells were filled with 0.1 ml of prepared concentrations for each mixture (sidr infusion 62.5 g/L and 1.5% hydrogen peroxide) and incubated the plates at 37 C for 24 hr. The susceptibility to the mixture was determined by measuring the inhibition zone around the wells for each concentration.[23]

RESULTS

The results of this experiment showed that *P.aeruginosa* has a great resistance to most antibiotics commonly used in hospitals, since the majority of isolates were resistant to more than four antibiotics and the prevalence of multidrug resistant *P.aeruginosa* (resistant to antibiotics belong to 3 different antimicrobial groups) was 35%. *P.aeruginosa* showed different susceptibility towards antibiotics. in this study study revealed presence of multidrug resistance (MDR) among *P. aeruginosa* that show high level of resistance Cefataxime (CTX:30mg), Ceftazidme (Ca:30mg). Gentamicin (CN: 10 mg) and Carbenicillin (Py: 100mg). while the *P.aeruginosa* isolate was sensitive to Norfloxacin (NOR:10mg), Tobramycin (TOB:10mg) and Amikacin (Ami:30 mg) (Table 1).

Table 1: The Sensitivity of *P.aeruginosa* to the Various Antibiotics

Strain	Antibiotics Susceptibility						
	CTX 30	CA 30	CN 10	Py100	NOR 10	TOB10	Ami30
P.aeruginosa	R(0)mm	R(0)mm	R(4)mm	R(10)mm	S(22)mm	S(16)mm	S (20)mm

(CTX 30mg): Cefataxime, (CA 30mg): Ceftazidme, (CN 10mg): Gentamicin, (Py 100mg): Carbenicillin, (NOR 10mg): Norfloxacin, (TOB 10mg) :Tobramycin and (Ami 30mg): Amikacin.

In this study (Table 2) (figure 1), the result that *P.aeruginosa* isolate was sensitive to mixture (sidr infusion 62.5g\L and 1.5% hydrogen peroxide) inhibition zone in well diffusion test was determined at 23mm according to[23]. While the *P.aeruginosa* isolate was resistance to when both either of them was used alone antibacterial solutions sidr infusion 62.5 g/L and hydrogen peroxide 1.5%.

Table 2: The Sensitivity of *P.aeruginosa* to the Antibacterial Solutions

Strain	Antibacterial Solutions		
	Sidr Infusion 62.5 g/L	Hydrogen Peroxide 1.5%	Mixture (Sidr Infusion 62.5g\L with Hydrogen Peroxide 1.5%)
P. aeruginosa	(R)8mm	(R)13mm	(S) 23mm

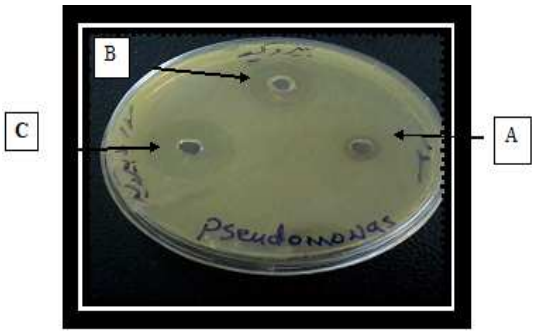

Figure 1: Sensitivity of *P.aeruginosa* to the Antibacterial Solutions. A: Sidr Infusion 62.5 g/L, B: Hydrogen Peroxide 1.5% and C: Mixture (Sidr Infusion 62.5g\L with Hydrogen Peroxide 1.5%)

DISCUSSIONS

Development of antibiotic resistance is often related to the overuse, and misuse of the antibiotics prescribed. Iraq is one of the developing countries where all types of antibiotics are sold over the counter, an attitude that encourages self-medication. The antibiotics susceptibility profile of *P. aeruginosa* isolates revealed high resistance for more than four antibiotics of different groups used in this study means multidrug resistance (MDR)[1]. Such high antimicrobial resistance is probably promoted due to selective pressure exerted on bacteria due to numerous reasons like non adherence to hospital antibiotic policy, and excessive and indiscriminate use of broad-spectrum antibiotics.[10] Hydrogen peroxide (HP) is an active agent that affects a wide range of organisms such as bacteria, yeast, fungi, viruses, and spores[24]. *Z. spina-christi* has recently been shown to have antibacterial, antifungal and antioxidant activities.[19] In this study, as shown in the first table that *P.aeruginosa* were sensitive to three antibiotics; Norfloxacin (22 mm), Tobramycin (16 mm) and Amikacin(20 mm). Second table we noticed that the mixture was highly effective against bacteria, which was Mixture (Sidr infusion 62.5g\L with hydrogen peroxide 1.5%) (23mm). When comparing the results between the two tables, we found the great the effectiveness of the new mixture of against *P.aeruginosa*.

CONCLUSIONS

The mixture of the sidr infusion 62.5g\L with 1.5% hydrogen peroxide was effective to inhibit completely *P.aeruginosa*. In this study we assessed the antibacterial effect of special solution made by a mixture of Sidr infusion with Hydrogen peroxide.

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Pseudomonas aeruginosa is a non sporulating, gram negative motile bacterium with a polar flagellum it possesses. It is ubiquitous microorganism widely distributed in soil, water and on living hosts and it can thrive in hot baths, diluted sterilizers, disinfectants, contact lenses and catheters.

The effect of mixture (sidr infusion 62.5 g/L and 1.5% hydrogen peroxide) on *P. aeruginosa* show remarkable results when compared to highly active sensitive antibiotics like Norfloxacin, Tobramycin and Amikacin.

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